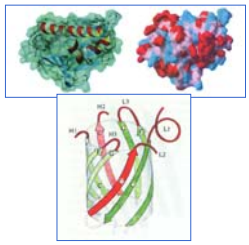
Allergenic Cross-Reactivity

Concepts, Patterns & Clinical Implications



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Overview

- **Cross-reactivities: Focus of considerable interest/investigation over last 10-15 yrs**
- **Coincides w/ significant scientific/clinical advances in allergy and clinical immunology**
  - ***In vivo* pathways/mechanisms**
  - ***In vitro* methodologies, Ag-Ab interactions**
  - **Allergen ID/characterization, Extract properties**
  - **Genetic/molecular biology developments**
  - **Clinical measurements of sensitivity and IT efficacy**

Overview

- **New developments and insights into the molecular basis of allergen cross-reactions**
  Diverse/novel approaches, methods and criteria
- **Shared vs. distinct Ag/epitope structures**
  Ag repertoires/pt profiles, Characteristic Ag groups
- **Relationships, similarities and relevance**
- **Medline: ~ 500 publications in last 10 years**
  Allergy/immunology + molecular/biochem journals

Challenges

- **How to acquire & maintain up-to-date information**
- **How to understand/interpret data and relationships**
- **How to establish cross-reactivity patterns pertinent to your patients and practice**
- **How & when to incorporate cross-reactivities into current testing and treatment regimens**

Concepts and Perspectives

- **Conservation of protein allergen structures**
- **Taxonomy/genetics vs. biochemistry**
- **Structure-function-activity relationships**
- **Clinical vs. immunochemical cross-reactivities**
- **Allergen-antibody binding requirements**

Key Elements

- **Patterns vs. # and ID of common components**
- **Sequence homology vs. structural similarity**
- **Major allergen definition, ID and importance**
- **Isoallergens and epitope diversity**
- **Continuous vs. discontinuous Ag structures**

Cross-Reactivity Perspectives

- **Clinical**
  - IgE specificities in mast cells, basophils and sera
- **Taxonomic**
  - Conservation and homology of genomes or sequences
- **Biochemical**
  - Allergen/antigen protein sequences and structures
  - IgE/IgG binding site conformations/topologies

Clinical Cross-Reactivity

- **Implied from patient specificity profiles**
  - Prick/intradermal skin test relationships
  - Total and allergen-specific IgE levels (CAP/RAST)
  - Exposures, challenges or IT with representative Ags
- **Strengths**
  - Comprehensive sens/spec patterns for individuals & gps
- **Weaknesses**
  - Cannot distinguish parallel/independent from shared Ags
  - Implications not always consistent w/ actual pt sens

Clinical Sensitivities

- **Single- vs. multiple-allergen sensitivities**
  - Not distinguished by history, skin test, sp IgE
  - Often confirmed by cross-wise IgE inhibition IAs
- **High CR relevance** — Pollens, dust mites
  Lower CR relevance — Fungi, insects, animals
- **Cross-reactive allergens = additive IT doses**
  - Diversity/mixes vs. Representation/individual Ags
  - Patient sens/exposures vs. Extract compositions

Taxonomic Cross-Reactivity

- **Consistent with phylogenetic relationships**
  - Common genus, order, family, subfamily, ...
  - Genetic mapping, Gene/protein sequence homologies
  - Complicated by presence of natural + designed hybrids
- **Strengths**
  - Patterns consistent w/ pt rxns in many (not all) cases
- **Weaknesses**
  - Epitopes may include unique, non-homologous regions
  - Sensitive to source material/extract/pt specificity diffs

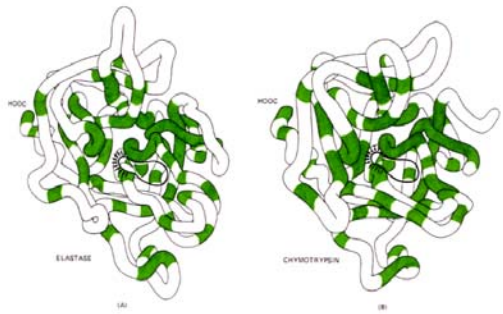
Biochemical Cross-Reactivity

- **Based on structural & immunochemical data**
  - Protein sequences/structures, Epitope/domain mapping
  - Compositional fingerprints (IEF, SDS-PAGE, CIE)
  - Immunoassays (ELISA, blot, CRIE) using human/animal Abs
- **Strengths**
  - Precise Ab specificities, Shared vs. unique 1^{o}, 2^{o}, 3^{o} regions
- **Weaknesses**
  - IgE specificities & CRs may differ from those found for IgG
  - Complicated by Ag isoforms, multivalent Ags, polyclonal Abs

Conservation of Allergen Structures

- **Similar genetic classifications and/or Similar protein functions**
- **Homology** — Total/local, Exposed/buried, 1°/2°
- **Isoforms** — Amino acids and sugar chains
  - Number + chemical nature of structural differences
  - Conservative vs. non-conservative substitutions
  - Carbohydrate chains (±), sequences and locations

Serine Protease Homology



Molecular Basis of CRs

- Requirements for orientations + interactions of antibodies and specific allergenic proteins
- Complementarity/spatial relationships between 3° (3D) Ag conformations and hypervariable regions of Ab molecules (F_{ab})
- Number + nature of contact regions essential to high-affinity, multi-point Ag-Ab binding

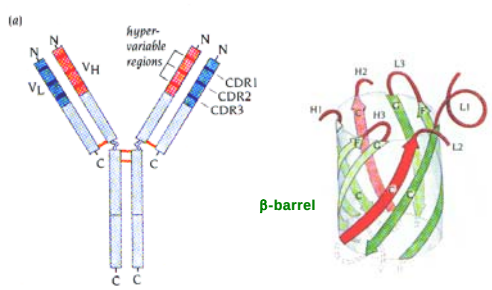
Allergen-Antibody Interactions

- Antibodies recognize precise arrangements of chemical groups on allergenic proteins
- Secondary/tertiary structures, cofactors, ionic + hydrogen bonds between Ag and Ab
- Includes linear (sequential) and non-linear (remote, conformational) allergen structures

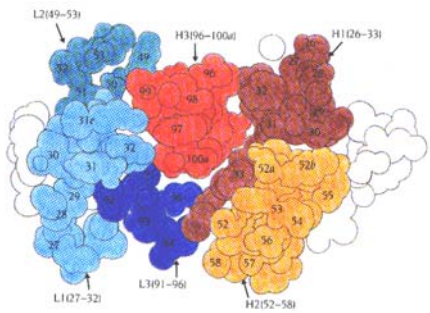
Allergen-Antibody Complexes

- Minimal & optimal combinations w/ binding strengths determined by overall fit/contacts
- Structures and interactions influenced by relatively minor changes in sequence/chem
- Complex formation sensitive to variations in patient specificity, raw material/extract composition, product stability/compatibility

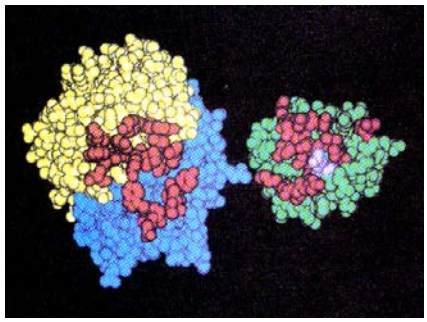
IgG/IgE Structure and F_{ab} Domain



F_{ab} V_HV_L Topography



MAb-Lysozyme Binding Site



General Patterns of Allergenic Cross-Reactivity

- **Same genus/tribe** — Strong cross-reactions
  Closely-related compositions and protein structures
- **Same subfamily** — Moderate cross-reactions
  Similar compositions with different/unique structures
- **Diff. subfamilies** — Low/no cross-reactions
  Distinct compositions/structures, minimal similarities

Tree Pollen Cross-Reactivities

- **Maple / Box elder** (genus Acer)
  Red cedar / Mtn cedar / Juniper (genus Juniperus)
  Cottonwood / Poplar (genus Populus)
  Birch / Alder / Hazelnut (family Betulaceae)
  Oak / Beech / Chestnut (family Fagaceae)
  Non-Juniperus cedars / Cypress (family Cupressaceae)
  Walnut / Hickory / Pecan (family Juglandaceae)
  Olive / Ash / Privet / Lilac (family Oleaceae)

Weed Pollen Cross-Reactivities

- **Giant/Short/False/Western ragweed** (genus Ambrosia)
  Dock / Sorrel (genus Rumex)
  Sage / Mugwort (genus Artemisia)
  Pigweed / Careless weed (genus Amaranthus)
  Lambs quarter / Mexican tea (genus Chenopodium)
  Marsh elder / Poverty weed (genus Iva)
  Scale / Saltbush (genus Atriplex)

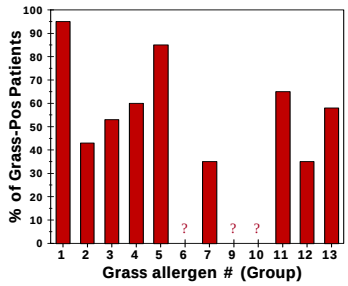
Grass Pollen Cross-Reactivities

- **Strong** — Blue/Fescue/Rye/Orchard/Brome (A)
  Timothy/Redtop (B)
  Sweet vernal/Canary/Reed canary (C)
  Cultivated oat/Velvet (D)
- **Moderate** — (A) vs. (B/C/D) ...
  Bermuda vs. Salt (E)
  Bahia vs. Johnson (F)
- **Low** — (A/B/C/D) vs. (E/F)
  (E) vs. (F)

Prominent Grass Pollen Allergens

Group	Activity or function	Mol wt (kd)
1	Beta-expansin	27-35
4	High MW alkaline glycoprotein	50-70
5	Starch granule-assoc. protein	27-35
7	Calcium-binding protein	8-12
10	Cytochrome c	11
11	Trypsin inhibitor (Soybean)	16-18
12	Profilin	12-14
13	Polygalacturonase	50-60

Prevalence of Positive IgE Reactions



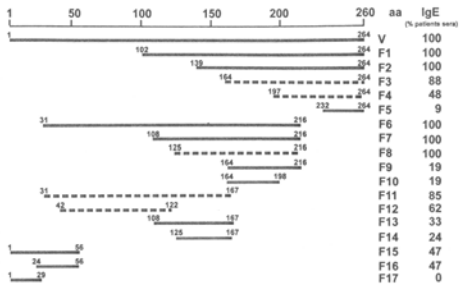
Group 1 Grass Allergens

- **Present in temperate + subtropical species**
  - Per. rye Lol p 1, Timothy Phl p 1, Bermuda Cyn d 1
- **Identical runs ≥ 10aa in 250aa Gp1 structures**
  - Tim 1 vs. Rye 1: 10 runs 10,12,14,16,18,20,22,24,27,31 aa; 22 aa diffs/247 = 9% 91% homology; 22 diffs = 11 conserv + 11 non-conserv
  - Ber 1 vs. Rye 1: 1 run 11 aa; 76 aa diffs/247 = 31% 69% homology; 76 diffs = 24 conserv + 52 non-conserv
- **Most Gp1 IgE epitopes** Non-homologous regions

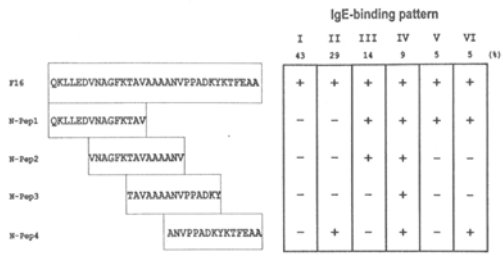
Group 5 Grass Allergens

- **Found in temperate but not subtropical sp.**
  - Perennial rye Lol p 5, Timothy Phl p 5, Velvet Hol l 5
- **rHol l 5 epitope map** Overlapping peptides
  - Schramm et al Clin Exp Allergy 2001; 31: 331-341
- **Fragments** May not ID discontinuous epitopes
  - Obs. in studies of Bet v 1, Der p 1 and Der p 2 structures
  - Short fragments (30 aa) Continuous sequ, low avidity
  - Long fragments Discont. sequ, higher avidity
  - rHol l 5 IgE epitopes 4 continuous + 5 discontinuous

Velvetgrass Gp 5 Allergen rHol l 5



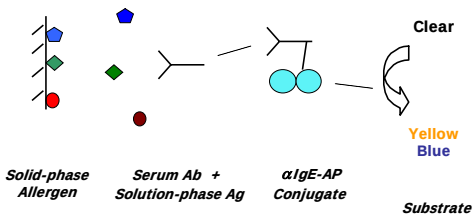
Velvetgrass rHol l 5 F16 Reactions



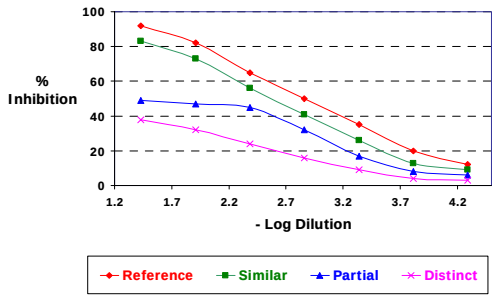
IgE Competitive Inhibition Assays

- **ELISA inhibition**
  - Quantitative / adsorbed native Ag extracts
  - Reveals degrees of compositional similarity
  - Estimates rel. potencies of test & reference Ags
- **SDS-PAGE immunoblot inhibition**
  - Qualitative/semi-quantitative w/ denatured Ags
  - Identifies common, partial & unique structures
  - Conformational epitopes (3^o) may be impaired

ELISA / Blot Inhibition Format

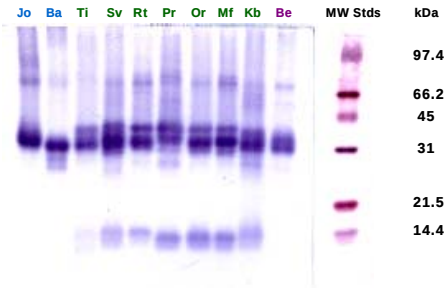


ELISA Inhibition Patterns



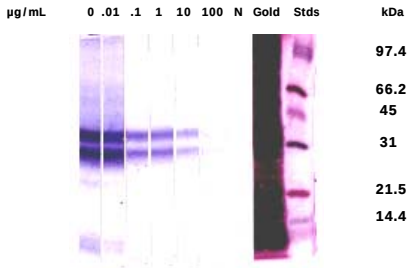
Grass-Pos IgE Immunoblot Profiles

Greer Subject # G001



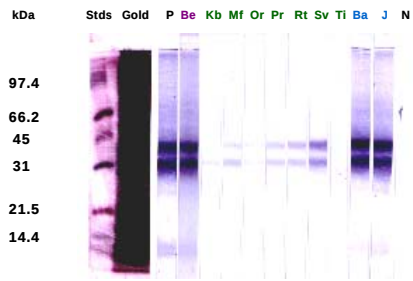
Blot Inhibitions Dose-Response

Pt G060 Immob: Tim Inhib: P. rye



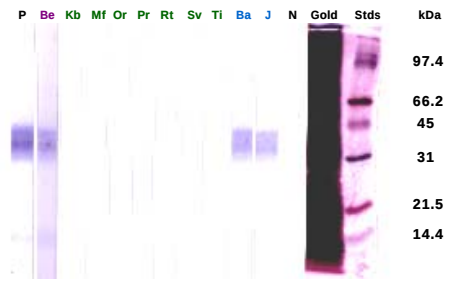
Blot Inhibitions 100 µg/mL protein

Pt G060 Immob: Tim Inhib: various



Blot Inhibitions 100 µg/mL protein

Pt G060 Immob: S. vernal Inhib: various



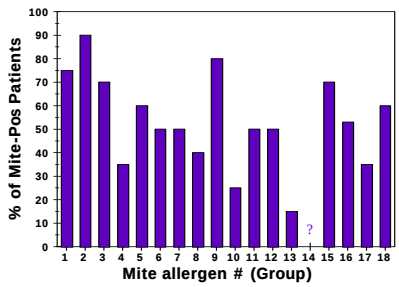
Dust Mite Cross-Reactivities

- **Near-complete betn Dermatophagoides spp.**
  - Shared (D. farinae, D. pter) + unique (mostly D. pter)
- **Moderate w/ related dust/storage mites**
  - Df/Dp/Euroglyphus magnei — family Pyroglyphideae
  - Acarus siro — family Acaridae
  - Tyrophagus putrescentiae — family Acaridae
- **Limited with other dust/storage mites**
  - Blomia tropicalis — family Echimyopididae
  - Lepidoglyphus destructor — family Glycyphagideae

Prominent Dust Mite Allergens

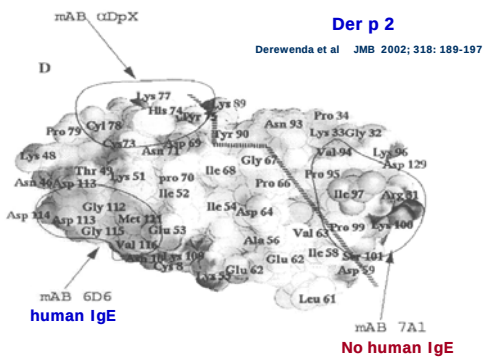
Group	*Activity or function*	*Mol wt (kd)*
1	Cysteine protease	25
2	Reproductive/molting protein ?	14-15
3	Trypsin-like serine protease	29-30
4	Amylase	56-60
6	Chymotrypsin-like serine protease	25
8	Glutathione-S-transferase	26
9	Collagenolytic serine protease	24-28
10	Tropomyosin	33

Prevalence of Positive IgE Reactions



Dust Mite Cross-Reactivities

- **Conserved structures within each Ag group**
  Same or different epitopes for IgE & IgG



Der 2 Isoallergens

- **Lys100 ⇨ Arg (conserv), ⇨ Thr/Glu (non-conserv)**
  Smith & Chapman Clin Rev Allergy Imm 1997; 27: 593-599
- **MAb binding to Der 2 Arg100, Glu100 vs. Lys100**
  - MAb αDpX — Arg100: slight ⬇ — Glu100: slight ⬇
  - MAb 15E11 — Arg100: slight ⬇ — Glu100: slight ⬇
  - MAb 7A1 — Arg100: moderate ⬇ — Glu100: signif ⬇
  - MAb 13A4 — Arg100: total ⬇ — Glu100: total ⬇
- **Ab specificity critical for Der 2 Ag quantitation**
  7A1 used as probe Ab in commercial Der 2 ELISA kits

Insect Cross-Reactivities

- Limited data, very few studies conducted
- Species-specific reactions are most common
- CRs observed among cockroach genera
  American (*Periplaneta*), German (*Blatella*), Oriental (*Blatta*)
  - Per a 1 / Bla g 1 — 70 % aa identity
  - Per a 7 (Tropomyosin) — 80 % homology w/ inverts
    Shrimp, mites, crustacea/mollusks
    45 % homology w/ verts
    Beef, chicken, pork, lamb

Animal Cross-Reactivities

- Animal serum albumins exhibit high CRs
  Cat, dog, human, horse, ...
- Major cat & dog allergens may CR in some pts
- Major mouse and rat urinary allergens may also CR in some pts
  Mus m 1 / Rat n 1 — Lipocalins
  65 % sequence homology

Mold Cross-Reactivities

- Partial-complete within a genus — Aspergillus
- Partial between some genera
  Alternaria, Stemphylium, Cladosporium, Curvularia, Stachybotrys
  - Alt a 10 / Cla h 3 — Aldehyde dehydrogenases
  - Alt a 11 / Cla h 6 — Enolase
  - Alt a 6 / Cla h 4 — Acidic ribosomal protein P2
  - Alt a 7 / Cla h 5 — S. cerevisiae protein YCP4
- None-partial within a genus — Penicillium

Food Cross-Reactivities

Group	*In vitro/sp IgE*	*Clinical*
Milk	Common	Common
Legume Grain Fish Crustacean/Mollusk Tree nut	Common	Uncommon
Egg-chicken	Occasional	Rare
Milk-beef		Uncommon
Tree nut-pollen	Occasional	Occasional

Food Cross-Reactivities

- Proteins from distant/unrelated taxonomies with similar functions
- Syndromes — Pollen-food — Birch/mugwort/fruits
  Latex-food — Banana/avoc/chestnut
- Shared allergens
  Plant defense/pathogenesis-related proteins
  Profilins
  CCDs — Cross-reactive carbohydrate determinants

Oral Allergy Syndrome (OAS)

- Pollen Ag sensitization linked to food allergy
  1st reported with birch pollen & apples ~ 50 years ago
- Pollens and foods share homologous allergens
  Taxonomically related in some (but not all) cases
  Sensitizations to pollens precede reactions to foods
  Pollens — Birch, mugwort, hazelnut
  Foods — Apple, celery, carrot
- Birch-celery — Bet v 1 (PR10) — Bet v 2 (profilin) — 46-60 kD
  Mug-celery — Bet v 2 (profilin) — 46-60 kD

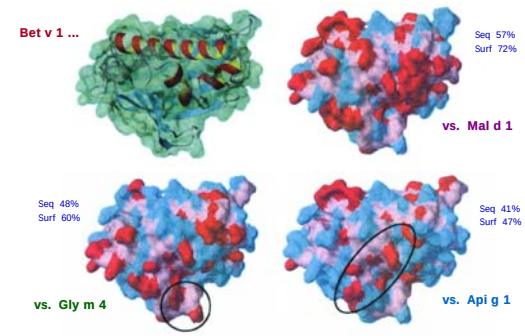
Pathogenesis-Related (PR) Proteins

- **Families** **Conserved, stable Ag structures**

PR2	β1-3 glucanases	Latex Hev b 2
	Barley, wheat, soybean, rice, mustard, cucumber	
PR3	Endochitinases	Latex Hev b 6.02 (N term)
	Banana, avocado, chestnut, kiwi, peach, rice, strawberry, citrus, potato, wheat, turnip	
PR4	Endochitinases	Latex Hev b 6.03 (C term)
	Potato, soybean, tobacco	
PR5	Antifungal	Mt cedar, cherry, apple, pepper
PR8	Chitinase	Latex hevamine, cucumber
PR10	Bet v 1 homologs	
PR14	LTPs	Lipid transfer proteins

PR10 Bet v 1 Family

- **Defense against bacterial & fungal pathogens**
  Major Ags shared by many fruits, vegetables, nuts, pollens
- **Homologs** 17-22 kd, 154-160 aa, common 2° struc
  Birch, alder, hornbeam, hazelnut, chestnut, oak pollens
  Apple, pear, cherry, soybean, celery, carrot foods
- **Isoallergens** Birch Bet v 1, Apple Mal d 1
  Single aa diffs sufficient to destroy CRs
- **CRs associated w/ higher % of skin test+ and symptom+ pts vs. Bet v 2 family (profilins)**



Jenkins et al J Allergy Clin Immunol 2005; 115: 163-170

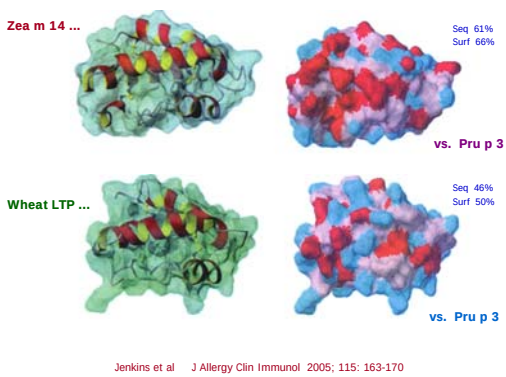
Bet v 1 Isoallergens

- **7 isoforms (1-5aa diffs) ID by IEF immunoblots**
  Akkerdaas et al Allergy 1995; 50: 215-220
- **HIgE/MIgG Ab reactivities to Bet v 1 isoforms**

pI	5.0	4.8	4.6	4.5	4.4	4.3	4.2
IgE JH	+	+	+	+	+	+	+
MAb 5H8	+	–	+	+	+	+	+
MAb 7F7							
MAb 9C11	+	–	–	+	–	+	+
MAb 11E12							
MAb 3C4	–	–	+	+	–	+	–

PR14 LTP Family

- **Inhibit bacterial and fungal pathogens**
  Independent sensitizations: resp (pollen) and oral (food)
- **Homologs** 9-10 kd, 91-95 aa, phys/chem resistant
  Mugwort, chestnut, sycamore, parietaria pollens
  Apple, peach, plum, apricot, soybean, corn, wheat foods
- **Pts w/ no birch or grass pollen sensitivities**
  ID by ST w/ plum: high [LTP], very low [Bet v 1] & [Bet v 2]
- **Anaphylaxis: 2X higher for PR14+ vs. PR10+ pts**
  LTPs concentrated in outer layer (peel), much less in pulp
  Severe reactions to some fruits attributed to LTPs alone



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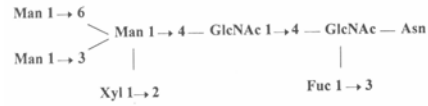
Profilins Bet v 2 Family

- Cytoskeletal, actin-associated, fertilization
  Sensitizations via pollens, CRs with fruits and vegetables
- Homologs 12-15 kd, 30-80% sequence homology
  Birch, alder, hornbeam, hazelnut pollens
  Apple, pear, peach, cherry, apricot, celery, carrot, potato, tomato, pumpkin foods and spices
- Minor (20%) panallergen CRs w/ Latex Hev b 8
- CRs associated w/ higher % of CAP/RAST+ pts vs. Bet v 1 family (PR10)

Cross-Reactive Carbohydrate Determinants (CCDs)

- Non-mammalian, immunogenic, stable, rigid



- Found in wide variety of allergenic materials
  Pollens and foods + insect venoms, shellfish
- High IgE binding, very low clinical relevance

Latex-Food Cross-Reactivities

Latex protein	*CR group or food*
Hev b 2	PR2 β1-3 glucanases
Hev b 3	Red kidney bean
Hev b 5	Kiwi
Hev b 6	PR3/4 Endochitinases
Hev b 7	Potato
Hev b 8	Bet v 2 family Profilins
Hev b 9	Enolases Alt/Clad enolase
Hev b 10	SODs Aspergillus Mn^{2+} SOD

Conclusions

- Knowledge of CR patterns/mechanisms continues to expand at a rapid pace
- Clinical/biochemical tests (+ taxonomy): Positive ID of many important CR allergens
- Incorporation in Dx/Rx practices effective but many CR relationships remain obscure
- Collaborative studies essential to identify, understand & manage clinically-relevant CRs

Selected References

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Q&A

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